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COMMUNICATIONS

AN INVESTIGATION INTO THE THEORIES ON THE FORMATION AND EXIT OF THE INTRA-OCULAR FLUIDS*

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FOR many years there has been controversy regarding the mechanism of formation of the aqueous humour. The intra-ocular fluid has in turn been regarded as a secretion, exudate, transudate and dialysate, and various arguments have been brought for and against the various concepts. It is proposed here to refer only very briefly to the main theories of aqueous humour formation as detailed critical investigations have been carried out by previous writers.

The Secretory Theory:—The theory of secretion demanded the presence of certain cells whose special function was to produce aqueous humour and support for this view has been obtained chiefly anatomically, by cytological and electrical evidence of cellular activity, and by the stimulation of secretion by the action of drugs such as eserine. The ciliary body was believed to be the active gland.

The Transudation Theory:—In the transudation theory, the aqueous was held to be produced by a simple process of filtration at the ciliary body to circulate through the eye from the posterior to anterior chamber and thence find its exit at the canal of Schlemm.

The Dialysation Theory:—In the theory of dialysation, investigators have been influenced by comparative chemical studies of the blood and aqueous, and on the appearance in the aqueous of foreign substances such as fluorescein when injected into the blood stream. They have maintained that the aqueous was in osmotic and hydrostatic equilibrium with the capillary blood of vessels within the eye.

More recent views are that the aqueous humour is a dialysate and this has been based on a study of the chemical equilibrium, the chemical constitution of the abnormal aqueous, the osmotic equilibrium and the variations in the intra-ocular pressure following osmotic variations in the blood. A great deal of this work has been done by Duke-Elder (1926-1936) and it is proposed briefly to summarise the lines of his investigations, and later on in the paper to discuss his conclusions.

It will be remembered that Duke-Elder has re-measured the pressure relationships in the vessels of the eye and these were found to be—

arterial pressure 75 mm. Hg.
venous pressure 23 to 24 mm. Hg (1 to 2 mm. above the intra-ocular pressure).

From these pressure relationships he concluded that fluid interchange was possible throughout the eye as a whole. The attraction of fluid back to the blood vessels was believed to be accomplished by the osmotic attraction of the plasma proteins " inasmuch as the venous pressure in the eye is normally higher than the intra-ocular pressure, a hydrostatic outflow is impossible."

The Chemical Equilibrium

I (*a*) *The distribution of the electrolytes.*—Before going on to a general discussion of the electrolytic equilibrium that exists between the intra-ocular fluid and the blood, it is proposed very

briefly to mention the Donnan theory of equilibrium (1911), because this will help in the explanation of the various relationships between blood and aqueous humour which are to follow. The following diagram represents two electrolytes Na R and Na Cl separated by a membrane (indicated by a vertical line).

Na	Na
R	Cl
(1)	(2)

If the membrane is impermeable for the anion R of a salt Na R (and also for the non-dissociated part of the salt Na R) but permeable for all the other ions and salts, then when equilibrium has been established the following condition will exist.

Na	Na
R	
Cl	Cl
(1)	(2)

The solution containing the impermeable anion to the left of the above membrane is known as " phase L^1," while that on the other side is known as " phase L^2."

Although in theory the formula for the equilibrium of the ions across the membrane should be expressed in terms of activities most writers have used the simple formula (No. 1) which is valid on the assumption that the activities of the ions are equal to their concentration in grams molecules per litre of solution and that the osmotic pressure is low.

$$\text{(No. 1)} \quad [\overset{+}{\text{Na}}]_1 \times [\overset{-}{\text{Cl}}]_1 = [\overset{+}{\text{Na}}]_2 \times [\overset{-}{\text{Cl}}]_2$$

where the brackets signify molar concentrations.

Thus the product of any pair of diffusible cations and anions on one side of the membrane is equal to the product of the same pair of cations and anions on the other side. On the side of the membrane containing the non-diffusible R $(Na)_1$ is the sum of—

(i) cations formed by the dissociation of Na R
and (ii) cations formed by the dissociations of Na Cl.

On the other side of the membrane $(Na)_2$ is the concentration of ions only in combination with Cl_2 and obviously these concentrations of $[Na]_2$ and $[Cl]_2$ must be equal. In the phase L^1 the concentrations $[Na]^1$ therefore exceeds $[Cl]^1$. In the phase L^2 $[Na]^2$ and $[Cl]^2$ must be equal. Since the products of the

concentrations are the same in both phases it follows that $[Na]^1$, which is greater than $[Cl]_1$ must be greater than $[Na]^2$ and that $[Cl]^1$ must be less than $[Cl]^2$.

i.e.:—

$$Na_1 > Na_2$$
$$\text{and } Cl_1 < Cl_2$$

This inequality in the distribution of electrolytes is frequently met with in biological fluids, and this phenomenon has been investigated in the aqueous humour. The ocular equilibrium has thus been demonstrated—

$\overline{P}$ = (Plasma protein)	
Na^+	Na^+
Cl^-	Cl^-
Plasma	Capillary wall — Aqueous humour

On the left of the capillary wall is the plasma containing among its other constituents sodium proteinate which is unable to permeate the capillary wall into the aqueous chamber and sodium chloride which freely traverses the membrane. To fulfil the Donnan equation when equilibrium is established, the product of the concentrations of sodium and chloride in the plasma must equal the product of the concentrations in the aqueous. Since there is a non-diffusible sodium salt of protein, in the plasma, it follows there must be more sodium on the plasma side of the membrane, and to equalise the concentration of diffusible ions, there must be more chloride on the aqueous side of the membrane. Thus—

$$[Na^+] \text{ of plasma} \times [Cl^-] \text{ of plasma} = [Na^+] \text{ of aqueous} \times [Cl^-] \text{ of aqueous}$$

$$\text{and } [Na^+] \text{ plasma} > [Na^+] \text{ aqueous}$$

$$\text{and } [Cl^-] \text{ plasma} < [Cl^-] \text{ aqueous}$$

In making comparative chemical studies of the blood and aqueous humour, difficulties have always been encountered with regard to the small volume of aqueous humour available. In 1924 Lehmann and Messmann (1924) noted that the concentration of chloride was higher in the aqueous than the blood, and they suggested that the Donnan equilibrium existed. Later, Duke-Elder

(1927, *a* and *b*) analysed a sample of aqueous humour that had been obtained from the eyes of horses immediately after their slaughter and compared this with the serum of a healthy horse. He found the relative concentrations of sodium and chlorine to be in millimols per litre—

Chlorine of aqueous	123
Chlorine of blood	103
Sodium of aqueous	121
Sodium of blood	145

Making the theoretical equation—

$$[Na]\ \text{aqueous} \times [Cl]\ \text{aqueous} = [Na]\ \text{blood} \times [Cl]\ \text{blood}$$

$$121 \times 123 = 145 \times 103$$

or

$$148{\cdot}83 = 149{\cdot}35$$

Duke-Elder therefore concluded that the aqueous was a dialysate.

(*b*) *Non-electrolytes.*—According to the Donnan theory, diffusible non-dissociated substances ought to be in equal concentrations in the aqueous humour and the blood. A summary of Duke-Elder's figures was—

	Aqueous humour		Blood	
	mgs. per 100 c.c. solution	mgs. per 100 grams water	mgs. per 100 c.c. solution	mgs. per 100 grams water
Non-protein nitrogen -	23·6	23·6	23·9	25·6
Urea - - - -	28·0	28·0	27·0	28·9
Creatinine - - -	2·0	2·0	2·0	2·1
"Sugar" - - - -	98·3	98·6	91·0	97·4
Amino-Acid - - -	2·9	2·9	3·5	3·7

Duke-Elder stated that a correction factor must be applied to aqueous humour and blood in comparing the distribution of diffusible constituents to allow for the difference in solid displacement due to the unequal mass of solute. Thus the proteins of the serum only appear in traces in the aqueous humour. A correction factor of 1·003 to the aqueous and 1·07 to the serum was applied. From his results it can be seen that with the exception of the amino-acid nitrogen which Duke-Elder considered "too variable a quantity to permit reliable deductions to be made from it when the method of collecting materials is borne in mind," these non-dissociated substances are equally distributed in aqueous humour and blood. He also compared the sugar in the aqueous and blood in the same animal, using three rabbits. The average reducing substance for the three rabbits was—

Aqueous humour	151	mgs. per 100 c.c.
Arterial plasma	158	,, ,, ,,
Venous plasma	133	,, ,, ,,

and his results confirmed his previous conclusions.

II. *Chemical Constitution of the Abnormal Aqueous.*—Concurrent salt and sugar estimations were made on rabbits before and after the formation of plasmoid aqueous and in each specimen of plasmoid aqueous formed, the percentage of protein was estimated. Duke-Elder maintained that his results showed no change in sugar content but that an alteration in the chloride content compatible with a dialysation took place.

III. *The Osmotic Equilibrium.*—The osmotic pressure of the aqueous humour has been studied by many investigators since Dreser (1892) determined the freezing point depression of the intra-ocular fluid of the cow in 1892. Duke-Elder compared the osmotic pressure of the blood and aqueous humour by direct measurement in the following manner. *The colloid osmotic pressure of the plasma*:—In the first place the difference in the osmotic pressure between the plasma and the aqueous humour was demonstrated by equilibrating the blood with the aqueous humour. This was measured with a micro-osmometer using a cellophane membrane impermeable to colloids, and the osmotic pressure of rabbit plasma was found to exceed that of the aqueous humour by about 20 mm. Hg. Different species of animals were studied and it was found that the excess of osmotic pressure of the plasma over the aqueous humour varied directly with the amount of protein present.

The osmotic pressure of the crystalloids.—The membrane employed in the previous experiment though impermeable to the colloids, allowed free permeation of the crystalloids. Variations in the distribution of the crystalloids was determined by estimating their concentration before and after equilibrium had been established, and this was done by electrical conductivity measurements, it being assumed that the measure of its conductivity under constant conditions was an accurate index of its salt concentration. His measurements showed that there was no alteration in the total concentration of the dissociated salts in the aqueous after time had been allowed for equilibration between aqueous and arterial plasma. He excluded any change in the concentration of any of the undissociated crystalloids by determining the concentration of sugar in the aqueous before and after the experiment. He concluded that " the osmotic pressure of the aqueous is equal to that of a dialysate of the blood in the arterial capillaries."

IV. *Relation between the Osmotic Pressure of Aqueous Humour and Blood.*—By assuming that the aqueous humour was

a dialysate, Duke-Elder has maintained that the pressure of the eye is preserved normally by a balance between the hydrostatic pressure in the capillaries and the difference in osmotic pressure between the aqueous humour and capillary plasma. He has contended further that the intra-ocular pressure may be made to vary by disturbing the equilibrium between the blood and the eye, and he has sought to demonstrate this by altering the osmotic concentration of crystalloids and colloids in the plasma. His experiments were roughly divided into the following three groups.

1. Alteration in the crystalloid content of plasma by the intravenous injection of

(*a*) Hypotonic solutions.
(*b*) Hypertonic solutions.

Using an injection of isotonic saline as a control.

2. Alteration in the colloid content of the blood by injection intravenously of 15 per cent. aqueous gum acacia.

3. Alteration in the osmotic pressure of the blood by first

(*a*) Bleeding an animal.

and (*b*) Replacing the volume removed by 6 per cent. gum acacia in Ringer, by 15 per cent. aqueous gum acacia, or by saline injections.

Experiments of a similar nature were also carried out on the "perfused eye." From these experiments he felt he had established that the changes in the intra-ocular pressure, apart from those caused by capillary blood pressure, were explained by the osmotic outflow and inflow of fluid, the eye sharing "in the general dehydration and water-logging of all tissues of the body which follows a change in the osmotic value of the blood."

Thus Duke-Elder has maintained that the aqueous humour has been proved to be a dialysate from the chemical analysis of normal and abnormal aqueous humour, its osmotic pressure, and finally, by the relationship which existed between the osmotic pressure of the blood and the aqueous. It was felt that several of these later experiments of Duke-Elder's on the osmotic relationship between blood and aqueous warranted a very careful investigation in view of some of his unorthodox findings in certain experiments. In the discussion a full review is given of the experimental work carried out by previous investigators on the interchange of fluid following an alteration in the crystalloid content of blood which is represented by Duke-Elder's first group. In the experimental section, experiments have been designed to include the other two groups. It was decided in repeating his experiments to pay particular attention to the variations in the total volume of blood and the tissue dehydration following the artificially produced variations in its osmotic pressure.

Finally, a critical survey is made of all the evidence that has been brought forward in favour of the aqueous humour being a dialysate.

Experimental

Cats anaesthetised by intraperitoneal injection of " nembutal " (sodium ethylmethyl butylbarbiturate, Abbot Laboratories, Montreal), 0·5 c.c. (0·03 grams) per kilogram of body weight, were the sole animals employed. A canula in the carotid or femoral artery was used for blood samples, while a canula in the jugular vein was used for the infusion.

Total Blood Volume.—Details of the methods employed have been given in a previous paper by J. D. Robertson (1935). Changes in the blood volume were determined by haemoglobinometry. The colorimetric acid haematin method of Newcomer (1919) was adopted. Mendel and colleagues (1916) also O. Robertson and Bock (1919) have carefully investigated and proved the accuracy of this method of determining the blood volume under conditions which included the present experiments.

TABLE I

Time		Haemoglobin per cent.
9.19 a.m.	Sample of blood withdrawn (3·5 c.c.)	= 100
9.19–9.30 a.m.	43 c.c. of 15 per cent. gum in water injected	
9.30 a.m.	2 c.c. sample withdrawn	= 69
9.35 „	„ „ „	= 63
9.40 „	„ „ „	= 59
9.45 „	„ „ „	= 57
10.0 „	„ „ „	= 57
10.15 „	„ „ „	= 57
10.30 „	„ „ „	= 57
11.0 „	„ „ „	= 66
11.30 „	„ „ „	= 61
12.30 p.m.	„ „ „	= 66
1.30 „	„ „ „	= 71
3.30 „	„ „ „	= 74
5.30 „	„ „ „	= 83
7.30 „	„ „ „	= 91

Experiment 1:—The interchange of the fluids after the intravenous injection of 15 per cent. gum acacia in water. Five cats were employed and below is given a protocol of one experiment. Weight of cat=3·34 kg.

Calculated blood volume 70 c.c. per kg.=3·34 × 70=234 c.c.

The blood volumes at various intervals after the injection of gum were calculated in the following manner. Immediately after: Let X be volume immediately after—

$$234 \times 100 = 69X + 3{\cdot}5 \times 100$$
$$= \frac{23400 - 350}{69} = 331 \text{ c.c., or } 99 \text{ c.c./Kg.}$$

Below in table II are recorded in detail the changes which took place in the blood volume following the intravenous injection of 15 per cent. gum acacia. It expresses the mean of five experiments. In the column for the haemoglobin percentage, allowance is made for the blood removed for samples.

TABLE II

Time.	Haemoglobin	Blood volume c.c./Kg.
	Per cent.	
Before - - - - -	100	70
Injection of 15 per cent. gum in water		
Volume given 13 c.c. per Kg.	(84	83 Theoretical)
Immediately after injection -	70	100 Actual volume
5 mins. after - - -	63	112
10 „ „ - - -	58	121
15 „ „ - - -	57	122
30 „ „ - - -	57	122
45 „ „ - - -	57	122
60 „ „ - - -	63	111
2 hours „ - - -	69	101
3 „ „ - - -	73	96
4 „ „ - - -	81	86
6 „ „ - - -	86	81
8 „ „ - - -	95	74
10 „ „ - - -	100	70
24 „ „ - - -	102	68

During the injection of 15 per cent. gum in water, there was an attraction of fluid from the fluid depôts throughout the body and at the end of the injection, fluid had entered the vascular system to the extent of 17 c.c. per kg. This attraction of fluid continued for another ten minutes, then for the next thirty-five minutes the blood volume remained constant at 60 per cent. above its normal. One hour after the injection, the blood volume was falling; between four and six hours after the injection the blood volume had fallen to its theoretical amount, the vascular system having discharged the volume of fluid attracted from the tissues; and ten hours after the injection, the blood volume had returned to normal.

That the kidneys are not essential initially in disposing of the extra fluid added to the blood was proved by repeating the above experiment after bilateral nephrectomy. A similar curve was obtained. Leathes (1896), however, has previously demonstrated that restoration of blood volume after intravenous injections, took place as rapidly in animals with bilateral nephrectomy as in intact animals.

In subsequent experiments it was endeavoured to follow, as closely as the published details would allow, the technique and procedure adopted by Duke-Elder but several inconsistencies became apparent.

(1) The anaesthesia employed by him was an ether induction then maintained by chloralose (a compound of equal parts of glucose and chloral hydrate). Dryer and Walker (1913) have shown that chloral hydrate in quantities sufficient to produce anaesthesia causes a rapid concentration of the blood with a corresponding fall in blood volume and rise in tissue fluid volume. Thus experiments which are designed to demonstrate the fluid interchange become vitiated when use is made of chloralose as an anaesthetic.

(2) His procedure in bleeding was described (1927*c*): "the femoral artery was opened and allowed to bleed until the blood-pressure had fallen to about one-half of its original height . . . . the volume of blood was made up by the intravenous injection of an osmotically equivalent solution of gum arabic in Ringer (or a 15 per cent. gum arabic solution or physiological saline) until the blood-pressure had reached its original height."

No details were given as to—

(*a*) rate of haemorrhage,
(*b*) volume of blood removed,
(*c*) volume of fluid injected,
(*d*) rate of injection of fluid,
(*e*) the time intervals between the observations,
and (*f*) there was no time tracing on the charts.

These details are of importance, for example—If the haemorrhage is a rapid one, less blood will require to be removed to reduce the blood-pressure to half, than if the blood is removed slowly.

Again, a smaller volume of gum can raise the blood pressure to normal if introduced rapidly into the vein.

(3) He has written that the "volume of blood was made up" by the intravenous injection of various solutions. J. D. Robertson (1935) has shown that during a haemorrhage there is a prompt and immediate transfer of fluid from the fluid stores throughout the body to the vascular system to make good the deficit in blood volume. This transfer of fluid is so immediate that if samples are taken of the blood as it is removed from the carotid, a steady dilution of the haemoglobin is seen. From experiments carried out on haemorrhages varying from 12 to 37·5 per cent. of the total blood volume, so rapidly did this transference of fluid from the reserves of the body take place that immediately the haemorrhage had ceased equilibrium in the blood volume had practically been established. Notable exceptions were in the cases where the haemorrhage exceeded 30 per cent. of blood volume, and in these the blood volume continued to increase beyond the normal level until death took place in about ten minutes. Although equilibrium in blood volume had been established immediately the haemorrhage had ceased, it was noted that the post-haemorrhage blood volume was not quite so great as the original blood volume. The difference was, however, small, the average blood volume being in the

TABLE III

Exp. No.	Vol. of blood removed as percentage of total	Weight in kg.	Duration of hæmorrhage (min.)	Fluid from tissues during hæmorrhage				Plasma removed (original plasma)	
				To circulating blood A	To hæmorrhage blood B	Total vol. A + B	Vol. per kg. per min.	Total vol. C.	Vol. per min. per kg.
79	12	2·76	2	19	1	20	3·6	18	3·25
72	16·5	4·5	5	43·5	1	44·5	2·0	37	1·64
71	22	4·34	5	36	1	37	1·7	42	1·94
77	25	3·12	5	24	4	28	1·8	38	2·40
73	28	3·4	6	33	3	36	1·7	49	2·40
74	30	3·4	6	29	3	32	1·6	55	2·70
75	32	3·26	10	84	12	96	2·9	67	2·06
78	37·5	3·70	14	57	20	77	1·5	72	1·40
							Mean = 2·1		Mean = 2·2

(1) Tissue fluid was entering vascular system during haemorrhage at 2·1 c.c./min./kg.
(2) Plasma fluid was removed by bleeding at average rate of 2·2 c.c./min./kg.

region of 64 c.c./kg. or almost 92 per cent. of the original blood volume. Table III from J. D. Robertson (1935) demonstrates the interchange of fluid observed.

It can be seen that the withdrawal of plasma by haemorrhage was at the rate of 2 c.c. per min. per kg. of body weight, while the entrance of fluid from the tissues and fluid reserves to the vascular system during the haemorrhage was 2·1 c.c. per min. per kg.

It was therefore decided to employ a uniform technique for all experiments and at the same time to follow in the main Duke-Elder's experiments. The animals were all bled 20 per cent. of their calculated blood volume (assuming the blood volume to be 70 c.c. per kg.) and at the rate of 2 c.c. per minute per kg. body weight. The intravenous solution equal in volume to the haemorrhage was injected at the rate of 4 c.c. per minute per kg. immediately the haemorrhage was finished.

Experiment 2:—The interchange of fluid after cats had been bled 20 per cent. of their blood volume followed by the intravenous injection of 6 per cent. gum acacia in physiological saline.

TABLE IV

Time	Blood volume per kg. (c.c.)
Before experiment - - -	70
20 per cent. of blood volume removed by haemorrhage, then an equal volume of 6 per cent. gum in physiological saline given intravenously.	
Immediately after - - -	90
5 mins. after - - -	94
10 „ „ - - -	95
15 „ „ - - -	95
30 „ „ - - -	94
45 „ „ - - -	91
60 „ „ - - -	88
2 hours „ - - -	87
3 „ „ - - -	87

It is seen that following a haemorrhage and the intravenous injection of an equal amount of 6 per cent. gum acacia in Ringer, the theoretical blood volume is exceeded.

Fluid is attracted from the tissues to the extent of twenty-five c.c. per kg. until fifteen minutes after the injection. Then fluid begins to flow back to the tissues but three hours after the injection the blood volume was still above normal (*i.e.*, the tissues were still dehydrated).

Experiment 3 :—The interchange of fluid after cats had been bled 20 per cent. of their blood volume followed by the intravenous injection of 15 per cent. gum acacia in water.

TABLE V

Time	Blood volume per kg. (c.c.)
Before experiment - -	70
20 per cent. of blood volume was removed, then an equal amount of 15 per cent. gum acacia injected intravenously.	
Immediately afterwards - -	88
5 mins. after - - -	91
10 „ „ - - -	98
15 „ „ - - -	96
30 „ „ - - -	97
45 „ „ - - -	92
60 „ „ - - -	91
2 hours „ - - -	89
3 „ „ - - -	87

TABLE VI

Time	Blood volume per kg. (c.c.)
Before experiment - - -	70
22 per cent. of blood volume was removed, then 0·9 per cent. NaCl given intravenously, in amounts equal to the blood removed by bleeding.	
Immediately afterwards - -	82
5 mins. after - - -	77
10 „ „ - - -	75
15 „ „ - - -	72
30 „ „ - - -	72
45 „ „ - - -	72
60 „ „ - - -	72
90 „ „ - - -	71
2 hours „ - - -	69
3 „ „ - - -	68

The haemorrhage and the intravenous injection of 15 per cent. gum acacia was followed by an attraction of fluid from the tissues. The blood volume reached its maximum ten minutes after the injection, then fluid gradually began to leave the vascular system for the tissues. At the end of three hours the blood volume was still above normal.

Experiment 4 :—The interchange of fluid after a 22 per cent. haemorrhage followed by the intravenous injection of 0·9 per cent. NaCl.

Immediately after the intravenous saline, the blood volume was greater than its theoretical normal, indicating the presence of dehydration. Fluid then began to leave the vascular system but only at the second hour did the tissues contain more fluid than normally, and even at the end of the third hour the blood volume was still 97·0 per cent. of normal.

Discussion

The Circulation of the Intra-ocular Fluids :—Intimately bound up with the various theories on the mechanism of the formation of the intra-ocular fluids is the question whether any circulation exists in the fluids of the eye or not. It has been differently stated that there exists a continuous through and through circulation and again that no circulation exists whatsoever. The idea of a stagnant aqueous was first put forward by Hamburger (1900-1923) and received support from Weiss (1904-1925) and Magitot (1917-1928). They maintained that the only movement present was an interchange of fluid and ions across the capillary membrane all around the eye. Adherents of the Leber school including Nuel and Benôit (1900), Seidel (1918-1920), Ulbrich (1907-1908) and Priestley-Smith (1927) believed that a through and through circulation existed with its origin at the ciliary body and its exit at the canal of Schlemm. It was only natural that these views on the movement of the aqueous should influence these investigators in their theories on the formation of the intra-ocular fluids; thus those who maintained the aqueous humour circulated, believed it was formed by secretion or transudation, while those who said the aqueous humour was stagnant, believed it was formed by a dialysis. But a third and intermediate view has been put forward among others by Wessely (1921-1929) and Friedenwald and Pierce (1931-1933). They held that although the aqueous humour was in chemical, osmotic and thermodynamical equilibrium with blood plasma, nevertheless a through and through circulation existed. The views of Duke-Elder, one of the strongest protagonists of the dialysis theory, on the circulation of the intra-ocular fluids are well known to readers of this journal.

Duke-Elder's monograph drew from Priestley Smith the criticism (1927) that he had not made his position regarding the circulation of the intra-ocular fluids clear. Duke-Elder (1927d) met this by the further statement—" I therefore conceive of the eye as containing a fluid in membrane equilibrium with capillary blood. As such it must be essentially stagnant. At the same time the evidence physiological and pathological seems to demonstrate that the existence of a circulation cannot be denied."

The rate of flow of the aqueous humour has been investigated by several observers and estimates of the order of 50 c.mm. per minute have been obtained, but by inaccurate experimental methods. Recently Friedenwald and Pierce (1932) have measured the rate of flow in dogs and found it to be about 1 c.mm. per minute. Their experimental work was supported by some excellent observations upon a child with very small lenses. In one eye the lens became dislocated into the anterior chamber. While under observation the lens slipped in front of the pupil and formed a ball-valve with the iris. The volume of aqueous was calculated in the anterior chamber and about $\frac{2}{3}$ of this volume disappeared in 30 to 45 minutes. They estimated the rate of flow of the aqueous to be 1·5 to 2·5 c.mm. per minute.

These above investigations would appear to have established that a through and through circulation exists within the eye.

The Theory of Dialysation:—The dialysis theory has received support from Magitot (1917), de Haan and van Creveld (1921), Hertel (1914-1921), Wessely (1921-1929), Duke-Elder (1926-1936), and Friedenwald and Pierce (1931-1933); but whereas Magitot, de Haan and van Creveld and Weiss considered that there was no movement in the intra-ocular fluids, the others believed a through and through circulation existed. Ridley (1930-1931) has criticised the latter views by pointing out that if the intra-ocular fluids drain away, then they cannot be stagnant, and the aqueous cannot be said to be in Donnan equilibrium with blood plasma. The laws defined by Donnan apply only to fluids which are stagnant. Further, quoting Adair (1930), he has maintained that the Donnan effect is true only in dilute solutions and could not be obtained where 7 per cent. of protein is present on one side of the membrane as in the case of plasma. The Donnan equation expressed in the form of activity cannot be determined in the presence of protein. Neglecting for the sake of argument this objection to the theory of dialysis it is proposed to analyse the arguments in its favour. These have been summarised.

1. There is no evidence of the expenditure of any energy or demonstration of any activity in the formation of the aqueous humour.

2. The aqueous humour is in chemical equilibrium with blood.

3. The chemical constitution of the abnormal or plasmoid aqueous is compatible with a dialysate.

4. The aqueous humour is in osmotic equilibrium with blood.

5. The pressure of the eye is preserved by a balance between hydrostatic pressure in capillaries and difference in osmotic pressure between the aqueous fluid and the capillary plasma.

1. *Expenditure of Energy*:—The upholders of the dialysate theory ought to maintain that the interchange of fluid occurs equally throughout the eye as a whole, but they invariably postulate a site of greater entrance and a site of greater exit, thereby admitting of specialised structures in the manufacture and removal of the aqueous humour. These admissions would appear quite incompatible with the theory of dialysis, and if it could be demonstrated that the site of the source of the aqueous did not allow a return flow of fluid it would go a long way towards pointing to an expenditure of energy in the formation of the aqueous humour. Friedenwald and Pierce (1931) have investigated the permeability of the ciliary epithelium and they have found that neither water, dyes nor electrolytes can permeate the membrane of the posterior chamber in the reverse direction. This irreversible permeability of the ciliary epithelium has demonstrated that the potential energy on the two sides of the membrane cannot be equal and that energy or work must be expended in transferring fluid to the aqueous side of the barrier.

Thus there is evidence that there must necessarily be an expenditure of energy in the formation of the aqueous humour.

2. *The Chemical Equilibrium.* (*a*) *Non-electrolytes.*—According to the theory of dialysis the chemical equilibrium postulates that the non-electrolytic constituents such as sugar, urea, non-protein nitrogen, uric acid of the aqueous humour and blood plasma should be distributed equally between the aqueous humour and blood plasma. Of recent years the analyses of Duke-Elder have been regarded as the most complete and most reliable. Duke-Elder found an equal distribution of non-electrolytes, but examination reveals some points of criticism. Thus his conclusions are based first on a comparison of a pooled sample of the aqueous humour of many slaughtered horses with serum from one healthy horse and then by three experiments on rabbits in which the sugar in the aqueous humour and blood was the only diffusible electrolyte determined. The surprising fact is that Duke-Elder, in the first experiment under such uncontrolled conditions as pooled aqueous from horses of unknown health and uncertain food intakes, and in the second series of experiments in the presence of emotional excitement and general sympathetic disturbance tending to induce an increased sugar, should have obtained such equal distributions between blood and aqueous humour. Contrary evidence has,

however, been produced by others. Ask (1927), de Haan, and Van Creveld (1921), and Adler (1930-1933) found less glucose in the aqueous than in the blood. Adler (1930-1933) and Anderson (1921) found less urea in the aqueous than in the blood. By far the most conclusive piece of work, however, on the chemical equilibrium has been carried out by Walker (1933) with technique and material far in advance of any hitherto. In all, eighty experiments were carried out on frogs, fowls, rabbits, dogs, cats and man, and the composition of the aqueous was compared with that of the plasma of the same animal. His results completely opposed Duke-Elder's hypothesis and left no doubt that, as judged by the distribution of non-electrolytes, the aqueous humour could not be a dialysate. A summary of his findings with regard to his analyses on the aqueous humour were:—

1. That in rabbits, dogs and man, the urea concentration averaged only 68 per cent. of that of the plasma.
2. That the uric acid concentration in fowls and man is only 66 per cent. of that of the plasma.
3. In so far as reducing substances are concerned, three of his five experiments on rabbits supported Duke-Elder's belief that these substances were distributed equally between aqueous humour and plasma; the results of twenty-seven other analyses principally on frogs, dogs and man showed a deficiency when compared with blood.

As urea, uric acid, and sugar are entirely filtrable and easily diffusible it is impossible to reconcile these analyses of Walker's with the theory of dialysis.

(*b*) *Electrolytes*.—Great stress has been laid on the unequal distribution of the electrolytes of the aqueous humour and serum by Duke-Elder in support of the theory of dialysis. In the pooled sample of aqueous from many horses and blood from one healthy horse these were—

	Aqueous (millimols per litre)	Serum (millimols per litre)
Cations		
Sodium	121·2	145·6
Potassium	4·8	5·1
Calcium	1·5	2·5
Magnesium	1·1	1·2
Anions		
Cl'_{III}	123·1	103·2
PO_{4II}	1·38	1·26
SO_4	1·8	1·7

He then applied the sodium and the chloride concentrations in the form of the Donnan equation—

$$[Na^+]_{Aq} \times [Cl^-]_{Aq} = [Na^+]_{blood} \times [Cl^-]_{blood}$$

or $121 \times 123 = 145 \times 103$

$148{\cdot}83 = 149{\cdot}35$

It must be realised, however, that the analysis of a pooled specimen of aqueous humour obtained from the eyes of many horses condemned to be slaughtered is being compared with serum from one healthy horse. The results can at the most be only approximate and the wonder is that such a good agreement was obtained. Closer examination of his figures, however, indicates discrepancies, The sodium ratio (R_{Na}) or

$$\frac{\text{Sodium of the serum}}{\text{Sodium of the aqueous}} = \frac{145}{121} = 1{\cdot}20$$

and the chlorine ratio or

$$\frac{\text{chlorine of serum}}{\text{chlorine of aqueous}} = \frac{103}{123} = 0{\cdot}83$$

Van Slyke (1926) has shown that in a Donnan equilibrium $R_{Na} = 1{\cdot}04$ and $R_{Cl} = 0{\cdot}95$.

More recently, however, Duke-Elder in collaboration with Davson and Behan (1936) has reinvestigated this problem and overcome the objections to the above experiment by comparing the aqueous humour of cats with its corresponding plasma. The results they obtained would appear to demonstrate that a Donnan equilibrium existed for the ions of sodium, potassium and chlorine. Thus in the experiment which represented the pooled serum and aqueous humour of four cats, the analysis showed:— (the figures in millimols per kg. of water):—

Na		K		Cl	
Aqueous humour	Serum	Aqueous humour	Serum	Aqueous humour	Serum
150	160	6·00	6·50	129	123

The concentrations are expressed in millimols per kg. of water.

Then $[Na^+]$ aqueous $\times$ $[Cl^-]$ aqueous $=$ $[Na^+]$ serum $\times$ $[Cl^-]$ serum

becomes $150 \times 129 = 160 \times 123$

$193{\cdot}5 = 199{\cdot}8$

The R_{Na} was 1·07 (the authors in error calculate this as 1·05), the R_k 1·08, and R_{Cl} was 0·95 which showed a satisfactory agreement with the theoretical of R_{Na} 1·04 and R_{Cl} 0·96.

Although Duke-Elder's figures would appear to show the existence of a Donnan equilibrium, it must be remembered that he himself has admitted that a through and through circulation exists within the eye. Is it then possible for a Donnan equilibrium to exist in fluids that are not stagnant? Ridley (1930) has gone into this question fully. He has maintained that the direct application of the theoretical relation of Donnan to the intra-ocular fluids by Duke-Elder is unjustified because the Donnan effect is only true in solutions that are dilute and stagnant.

Further, a study of the distribution of only the sodium, potassium and the chloride in the blood and aqueous does not take into account the distribution of other anions and cations, and these must also conform in some manner to the Donnan equation. Analyses of these constituents by other experimentalists do not support a Donnan equilibrium. Thus the phosphate concentration of the aqueous has been found by Walker (1933) to be less than half that in the plasma, and by Tron (1928) to be 55 per cent. of that in plasma. In addition, Tron (1928) found the sulphate concentration of the aqueous was only 40 per cent. that of the plasma and that the potassium ratio was 1·61 (the potassium ratio of a dialysate is 1·06). Heubner and Meyer-Bisch (1926) found the sulphate of the aqueous to be half that of an ultrafiltrate of blood.

3. *Chemical Constitution of the Abnormal Aqueous.*—Another argument put forward by Duke-Elder (1927, *a* and *b*) in favour of the dialysate theory was the alteration that took place in the concentration of chlorides following the formation of plasmoid aqueous after paracentesis of the cornea. Concurrent salt and sugar estimations were made on rabbits before and after the formation of plasmoid aqueous, and in each specimen of plasmoid aqueous formed the percentage of protein was estimated thus:—

Aqueous removed in c.c.	Approx. % protein in plasmoid aqueous.
0·1	1·0
0·2	1·8
0·25	2·0
0·32	2·5

And it can be seen that the percentage of protein in the plasmoid aqueous increased in proportion to the amount of aqueous humour that had been withdrawn. He reported his sugar estimations in grams per 100 c.c. in the following way:—

Protein grams. per cent.	Normal aqueous	Plasmoid aqueous	Difference
1	0·143	0·148	0·005
1·8	0·155	0·173	0·018
2	0·173	0·175	0·002
2·5	0·165	0·172	0·007

In view, however, of the proportion of colloid constituents appearing in the plasmoid aqueous, it would appear that a correction factor should be employed in order the more accurately to compare the distribution of the constituents. Thus for :—

1 per cent. protein the factor is 1·01
1·8 per cent. ,, ,, ,, 1·02
2 per cent. ,, ,, ,, 1·02
2·5 per cent. ,, ,, ,, 1·03

The corrected figures then become :—

Normal aqueous	Plasmoid aqueous	Difference	Per cent. increase
0·143	0·149	0·006	0·004
0·155	0·176	0·021	0·014
0·173	0·178	0·005	0·003
0·165	0·177	0·012	0·007

So that the sugar showed a percentage increase of ·003 to ·014. Duke-Elder reported his salt estimations in g. NaCl. per 100 c.c. in the following way :—

Protein per cent.	Normal aqueous	Plasmoid aqueous	Difference
1	0·641	0·600	0·041
1·8	0·597	0·561	0·036
2	0·680	0·536	0·144
2·5	0·500	0·421	0·079

But by making an alteration for solid displacement as I have shown for the sugar, these results became :—

Protein	Normal aqueous	Plasmoid aqueous	Difference	Per cent. increase of
1	0·641	0·606	0·035	0·005
1·8	0·597	0·572	0·025	0·004
2	0·680	0·547	0·133	0·019
2·5	0·500	0·433	0·067	0·013

So that the salt showed a percentage decrease varying from ·004 to ·019.

With a sugar percentage increase varying from ·003 *to* ·014 *and a chloride percentage decrease varying from* ·004 *to* ·019, *it seems difficult to reconcile these results with Duke-Elder's conclusions.*

(Monograph p. 36). " It is thus seen that after paracentesis the colloid content of the plasmoid aqueous is increased and that it is increased in proportion to the extent to which anterior chamber has been evacuated. . . . *Coincidentally with this, the anions (chloride) show a diminishing concentration, while the non-ionized diffusible substances (sugar) remain unchanged.*" Later on in his monograph (p. 70) he has stated :—"Again when the permeability of the membrane to colloids is increased the plasmoid aqueous then formed, containing as it does a greater proportion of protein molecules, and therefore differing less profoundly from the plasma and so involving the existence of less powerful stresses, changes its composition in terms of the thermo-dynamical postulates we are considering the anions (chloride) relatively decrease and the cations increase *pari passu* with the increase in concentration of colloids." These are conclusions which are at variance with his own results, for the anions (chloride) although they do decrease do not do so *pari passu* with the increase in concentration of the colloids, and no analysis on the cations is recorded.

4. *The Osmotic Equilibrium* :—In an *in vitro* experiment, Duke-Elder by electrical conductivity measurements found that there was no alteration in the total concentration of the dissociated salts in the aqueous after time had been allowed for equilibration between the aqueous and plasma, thereby proving that the intra-ocular fluid was isotonic with blood. From these observations he concluded that a further argument had been brought forward in favour of the theory of dialysis. Gamble and McIver (1928) and Gilman and Cowgill (1931-1933) have studied the osmotic relations of blood and glandular secretions. They found that the gastric juice, hepatic bile, and pancreatic juice were all isotonic with blood, and that whereas the alimentary glands are unique in the

production of their characteristic secretions, the total concentration of their constituents was determined by a process in common —namely, the osmotic equilibration with the blood. After widely changing the osmotic pressure of the blood by hypertonic and hypotonic solutions, parallel variations were brought about in the osmotic pressure of these secretions. Thus, if Duke-Elder's deduction necessarily followed, then gastric juice, pancreatic juice, and hepatic bile are also dialysates, for all these secretions are isotonic with blood plasma.

He also maintained he had excluded any change in the concentration of any of the undissociated crystalloids by finding the sugar content of the aqueous unchanged after the experiment. But as Walker pointed out, although in three experiments out of five in rabbits, the sugar content of the aqueous and the blood were the same, nevertheless, in twenty-seven other experiments on other animals including frogs, dogs and man, the aqueous humour sugar content showed a deficiency when compared with blood. It is thus possible that Duke-Elder was unfortunate in his choice of animal and was misled by a chance agreement in the sugar content of aqueous and blood, apparently not an uncommon finding in the rabbit.

Duke-Elder's experiments upon the osmotic equilibrium of the aqueous humour prove no more and no less than that the aqueous falls into line with the cerebro-spinal fluid, gastric juice, pancreatic juice and hepatic bile in having a characteristic chemical composition and being in osmotic equilibrium with the circulating blood.

5. *The Relation Between the Intra-ocular Pressure and the Osmotic Pressure of Plasma.*—There can be no doubt that in some manner the osmotic pressure of the blood affects the intra-ocular fluid in the same way as it does all the other fluid secretions or transudates throughout the body. A more exact relationship between the intra-ocular pressure and the osmotic pressure of the blood has been attempted by Duke-Elder (1927-1928*c*). By assuming that the aqueous is a dialysate he has maintained that the pressure of the eye is preserved normally by a balance between hydrostatic pressure in the capillaries and the difference in osmotic pressure between the aqueous humour and capillary plasma. He has further contended that the intra-ocular pressure may be made to vary by disturbing the equilibrium between the blood and the eye, and he has sought to demonstrate this by altering the osmotic concentration of crystalloids and colloids in the plasma. So important are the deductions he has drawn from these experiments that it is proposed to study them in detail.

A. *Alteration in the Crystalloid Content of the Plasma by the Intravenous Injection of Hypotonic and Hypertonic Solutions*

Using Isotonic Saline as a Control.—The actual records are here reproduced below, showing the curves of the intra-ocular arterial and venous pressure following the various crystalloid injections along with Duke-Elder's comments.

(1) Isotonic solutions (*Brit. Jl. of Ophthal.*, 1926, Vol. X, p. 9), Fig. 1.

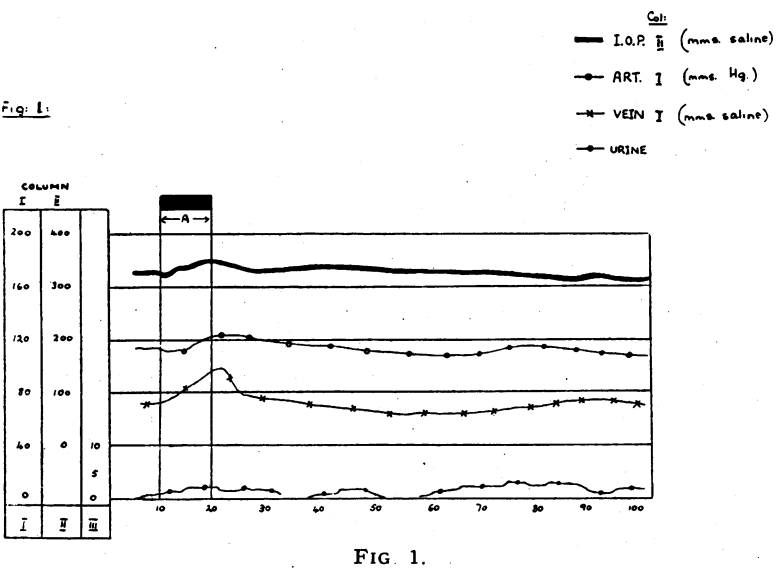

FIG. 1.

Control experiment. Injection of normal saline. Cat: female: 3,200 gms.: chloralose. The ordinates represent pressures as in Fig 1; the abscissae time in mins. During interval A, 30 c.c., 0·9 per cent. saline injected intravenously.

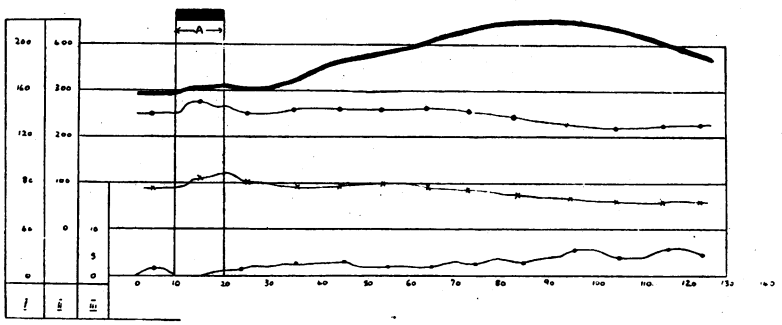

FIG. 2.

Hypotonic injection Cat: female; 2,800 gms.: chloralose. The ordinates represent pressures, read and graphed as in Fig. 1; the abscissae time in mins. During interval A, 25 c.c., 0·3 per cent. saline injected intravenously.

" The effect therefore of an isotonic injection is very small; it is purely mechanical and varies directly with the amount injected; and the intra-ocular pressure follows closely the curve of the blood pressure."

The Hypotonic Injection.—(*Brit. Jl. of Ophthal.*, 1926, Vol. X, p. 10), Fig. 2. 0·3 per cent. saline was injected.

" The intra-ocular pressure during the injection rose with the blood pressure, and immediately afterwards showed a tendency to decrease with it, which, however, was soon replaced by a rise which became much steeper 15 minutes after the injection, and

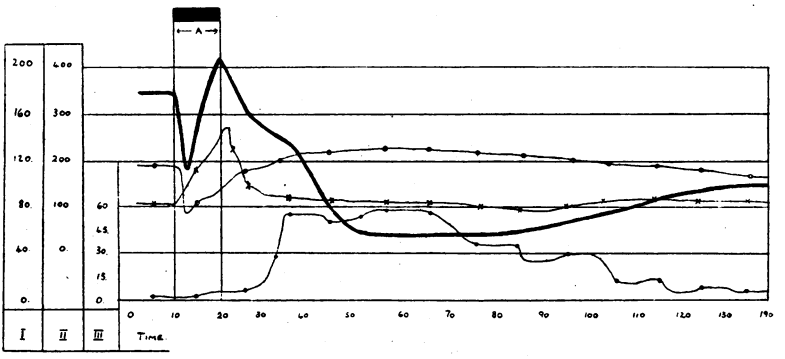

FIG. 3.

Hypertonic injection. Cat: female: 3,600 gms.: chloralose. The ordinates represent pressures read and graphed as in Fig. 1; the abscissae, time in mins. During interval A, 15 c.c., 30 per cent. saline injected intravenously.

attained a maximum of 450 mgms. of saline in 75 minutes, whereafter it began to fall. . . . No appreciable volume change could be made out " (Blood volume estimated indirectly by cell-count and haemoglobin).

The Hypertonic Injection.—(*Brit Jl. of Ophthal.*, 1926, Vol. X, p. 10). Fig. 3. 30 per cent. saline was injected.

" The intra-ocular pressure during the injection fell at first rapidly and profoundly (345 mm. saline to 180); thereupon it rose rapidly until at the end of the injection it had exceeded its initial value and had reached a peak of 415 mm. saline. After the injection it fell rapidly until in 30 minutes it had attained the level of 50 mm., about which figure it remained for about 40 minutes when it began to rise again. 30 minutes after completion of injection the blood volume had been increased by (approximately) 30 per cent. Then proceeding to a general summary of the mechanism of action Duke-Elder said (1926*a*)—" A study of the nature of the pressure curves and a comparison of the varying effect of the injection of different strengths of solution at different

rates by different routes, demonstrate clearly that there are two distinct influences at work involving two distinct responses, the first a rapid immediate change; following closely and depending upon the variation in part of the arterial in larger part of the venous pressure, and therefore presumably entirely of the (immeasurable) capillary pressure; the second, a later, more gradual, less variable change, completely independent of any blood-pressure variation differing from it in time of incidence, in rate of development and usually in direction." Duke-Elder thus felt that he had established that the changes in the intra-ocular pressure, apart from those caused by capillary blood-pressure were explained by the osmotic outflow and inflow of fluid, the eye sharing the processes of dehydration and water-logging similar to other tissues, for he had shown—

(1) When the concentration of crystalloids in the blood was raised by the injection of hypertonic fluid, the intra-ocular pressure fell because fluid was being attracted into the blood stream.

(2) When the concentration of crystalloids in the blood was lowered by the injection of hypotonic fluids, the intra-ocular pressure rose because fluid was being discharged from the blood stream.

The regulation of the blood volume and the fluid interchange between the blood and tissues after the intravenous injection of crystalloids of various percentages has interested many experimentalists since Brasol (1884) noted the rapidity with which the blood volume increased and fell after the intravenous injection of large quantities of sugar. Among others who have studied this problem are Leathes (1895), Bogert, Underhill and Mendel (1916) and Boycott (1913) and Boycott and Douglas (1914). Leathes also made observations upon the osmotic pressures of blood serum and lymph in relation to the injections. The main facts disclosed by their studies were:—

A. *Very Hypertonic Solutions.* 1. The increase in volume of the blood immediately after the intravenous injection of very hypertonic crystalloids (33 per cent. saline or 50 per cent. glucose) was marked and greatly in excess of that caused by the volume of the injected fluid.

2. The increase in volume took place during the process of the injection so quickly that the time taken for the transfer of fluid from the tissue was inappreciable. Osmotic equilibrium between lymph and blood was established as soon as the injection was stopped and thus the transfer of fluid from the tissues to the blood had also ceased then, since the force attracting the fluid was no longer present. So that immediately the injection was stopped, the volume of blood stopped increasing.

3. The moment the injection stopped, the volume of blood

began at once to diminish, ridding itself of the excess fluid and at the end of half-an-hour the blood volume was normal.

II. *Isotonic and Hypotonic* (0·3 *per cent. NaCl*) *solutions.*—Volumes given were 70 c.c. per kilo to 35 c.c. per kilo, or amounts equal to or about half that of the blood volume.

During the injection of isotonic and hypotonic solutions fluid began to leave the blood vessels for the tissues. It would be expected that the hypotonic solutions would leave much more rapidly than did the isotonic, but such was not the case. The difference was relatively small and as often as not, the isotonic fluids left the blood vessels more quickly than the hypotonic. Half-an-hour after the injection had been completed, however, more hypotonic fluid remained in the blood vessels than did the isotonic. This is a peculiar fact and Leathes has commented on it in the following words:—

"It is possible that hypotonic solutions exert some influence on the vasomotor system as Dr. Starling has suggested to me, from observations of his on venous pressures: he found the venous pressure to be raised by hypotonic injections as by isotonic, but for a much shorter time. This observation may explain perhaps the fact that Cohnheim's plethora experiments in which 0·6 per cent. NaCl was used, gave him a smaller rise of venous pressure than that found by Bayliss and Starling working with 1 per cent. NaCl solution. Perhaps, therefore, we may suppose that the effect of lowering the osmotic pressure of the blood is to increase the total capacity of the vessels and for this reason the injected fluid was not expelled so rapidly as would have been expected."

In view of these observations, and in particular those of Leathes, on the fluid traffic following the intravenous injection of varying strength solutions, re-examination of Duke-Elder's data shows that there is a difference between his findings and those of Leathes. Duke-Elder stated he found:—

(1) No appreciable blood volume change was made out when hypotonic solutions were injected.

(2) Half-an-hour after the completion of the injection of hypertonic solutions, the blood volume was increasing by 30 per cent. owing to dilution by tissue fluid.

The Intra-ocular Pressure after Hypertonic Injections.—In Fig. 4 the dotted line A.B.C.D. represents diagramatically what should be the curve of the intra-ocular pressure after the intravenous injection of hypertonic saline if the intra-ocular fluids were dialysates and the eye shared with the tissues the processes of dehydration and water-logging. For comparison, in heavy line, there is reproduced from Fig. 3 the actual curve Duke-Elder obtained after intravenous hypertonic saline.

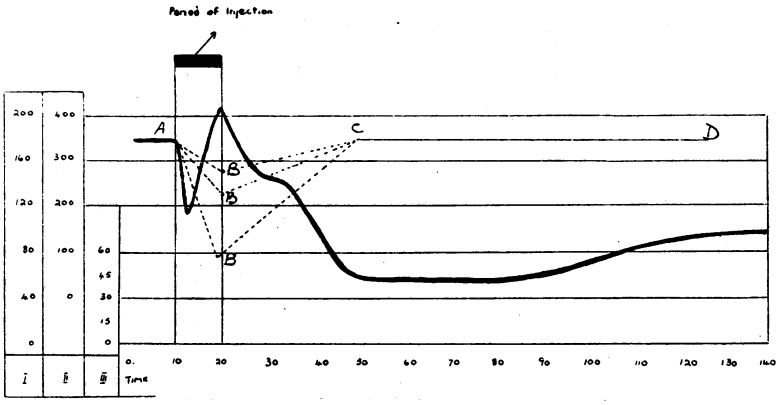

FIG. 4.

A B is fall due to the dehydration during the injection of hypertonic saline.

B C is the rise in pressure due to the pouring back of fluid from the blood stream, the blood volume, and tissue fluid returning to normal in 30 mins.

The position of the point B is of course indeterminate for it will depend upon a mean of the blood pressure effect, and capillary dilatation, with the amount of dehydration. The important point is that within half-an-hour (at C) the normal intra-ocular pressure should have been obtained, because blood pressure and capillaries are normal, and the fluid content of the tissues has returned to normal.

I.O.P.—Copied from Fig. 3. Duke-Elder's curve of the intra-ocular tension after hypertonic saline.

The Intra-ocular Pressure after Hypotonic Injections.—If the aqueous humour were a dialysate, then the curves of the intra-ocular pressure after the intravenous injections of hypotonic and isotonic solutions should be similar, as Leathes and others have shown that the discharge of fluid from the blood vessels is identical, or if anything the hypotonic fluid is retained intravascularly to a greater extent. Re-examination of the two graphs, Fig. 1 and Fig. 2 of Duke-Elder's after the isotonic and hypotonic injections, demonstrates only too clearly how wide apart are the variations in the intra-ocular pressure. This becomes all the more apparent when it is realised that Duke-Elder injected isotonic and hypotonic saline at only 9 c.c. per kilo, when previous observers had given as much as 70 c.c. per kilo.

It may be concluded, therefore, that from Duke-Elder's own experimental results, after crystalloid injections, proof has been established that the aqueous humour cannot be a dialysate.

B. *Alteration in the Colloid Content of the Blood by the Intravenous Injection of Gum Acacia.*—Duke-Elder carried out this experiment in the following manner (1927*c*). A rabbit was given 30 c.c. of 15 per cent. gum acacia intravenously and the intra-ocular pressure was read on a Schiötz tonometer. He found that

the intra-ocular pressure fell and remained reduced for over forty-eight hours. A reproduction of his chart is given below.

It may be assumed from this chart that the lowest intra-ocular pressure was obtained ten-and-a-half to eleven hours after the injection of gum when the intra-ocular pressure had fallen to 5·5 mm. Hg.

At the end of forty-eight hours although the tension was rising, the normal level had not been reached.

Fig. 6 represents graphically the changes that were effected in the blood volume after the intravenous injection of 15 per cent. aqueous gum acacia (Experiment No. 1). For comparison there

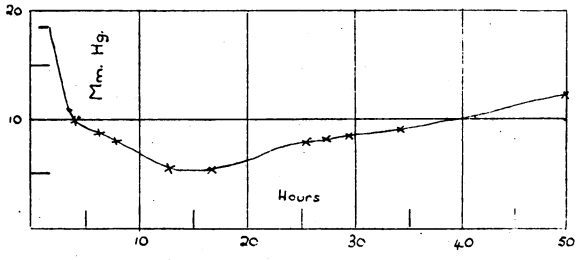

FIG. 5.

Variations of the intra-ocular pressure on increasing the colloid osmotic pressure of the blood.
30 c.c. 15 per cent gum arabic injected intravenously at second hour curve of intra-ocular pressure read on a Schiötz tonometer (cocaine) and transposed into mm. Hg. The crosses denote the time of reading (rabbit).

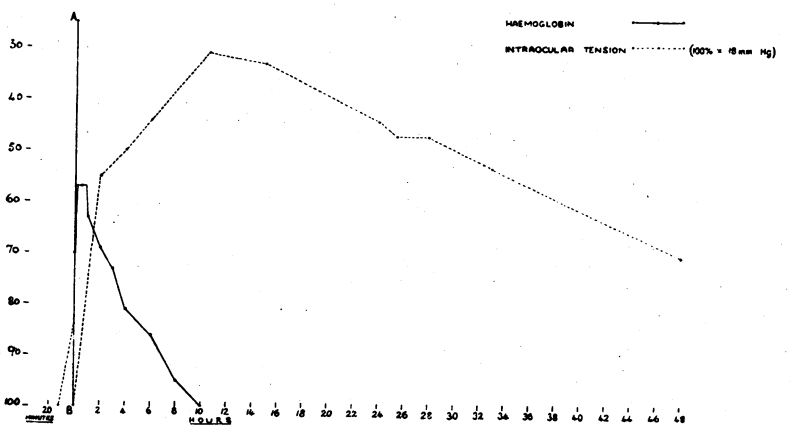

FIG. 6.

are also reproduced the changes which Duke-Elder found took place in the intra-ocular pressure after the injection of this strength gum acacia (Ref. Fig. 5).

The ordinates represent the volume of blood which is proportional to the dilution of haemoglobin, and the intra-ocular pressure. 100 per cent. in the intra-ocular pressure was determined by Duke-Elder in his experiment as being 18 mm. of mercury. The abscissae represent the intervals of time between the observations. The line A.B. indicates the end of the injection of gum and to the left of this line a dotted line indicates the duration of the injection and also the theoretical percentage of haemoglobin expected after the addition of gum (*i.e.*, if the original volume of blood is 70 c.c. per kg. the addition of 13 c.c. of gum per kg. should theoretically reduce the haemoglobin from 100 per cent. to 84 per cent.). The transfer of fluid from the tissues to the blood stream ceased ten minutes after the injection and three-quarters of an hour later fluid began to leave the vascular system. In ten hours the blood volume was normal, at which time the fluid content of all the tissues previously dehydrated must have returned to normal. That the kidneys are unimportant initially in disposing of the extra fluid attracted to the blood stream was proved by repeating the above experiments after bilateral nephrectomy when a similar curve was obtained. Leathes, however, had previously demonstrated that restoration of the blood volume after intravenous injections took place as rapidly in animals with bilateral nephrectomy as in intact animals. The intra-ocular tension as determined by Duke-Elder, however, fell steadily—it was falling at the end of an hour when fluid no longer was leaving the tissues, and it reached its lowest level ten-and-a-half hours after the injection at a time when the blood volume had returned to normal and all the transferred fluid had returned to the tissues. Thus the intra-ocular tension was lowest at a time when the blood volume was normal, and the tissues possessed a greater fluid content than normal by an amount equal to 13 c.c. per kg. of body weight.

Once again this experiment has demonstrated how the aqueous does not behave like a dialysate.

C. *Experiments after Haemorrhage.*—After bleeding an animal, Duke-Elder injected infusions of 6 per cent. gum in Ringer, 15 per cent. gum in water, and normal saline, and he noted the respective variations produced in the intra-ocular pressure.

i. *Intravenous Injection of 6 per cent. Gum Acacia in Ringer.*—In this experiment (Fig. 7) Duke-Elder found that the intra-ocular pressure fell coincidentally with the blood pressure during the haemorrhage, then rose with the blood pressure back to normal following the intravenous injection. When the interchange of fluid (Experiment No. 2, Table IV, p. 413), was studied after a similar experiment it was found that the theoretical blood volume was not maintained. After the intravenous gum injection, fluid had been transferred to the blood stream from the tissues to the

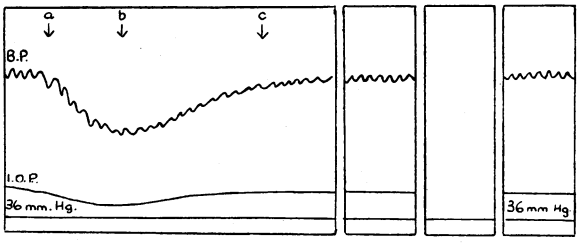

FIG. 7.

The variation of the intra-ocular pressure on altering the blood-pressure and the composition of the blood, the osmotic conditions remaining constant.
The upper tracing the blood pressure: the lower the intra-ocular pressure. At (*a*) the femoral artery opened; at (*b*) the bleeding stopped and injection of physiological saline and gum arabic commenced, and continued until (*c*). The intra-ocular pressure follows approximately the blood pressure. (Cat.)

extent of twenty-five c.c. per kg. up to the fifteen minutes after the injection. After this, fluid began to flow back to the tissues, but three hours after the injection the blood volume was still above normal (*i.e.*, the tissues were still dehydrated). It would follow therefore from this experiment that the aqueous does not behave like a dialysate, for if it did the curve of the intra-ocular pressure would have reached its minimum ten minutes after the injection and remained at that level for another twenty minutes. At the end of three hours the intra-ocular pressure should still have been below normal, because the vascular system still contained an excess of fluid.

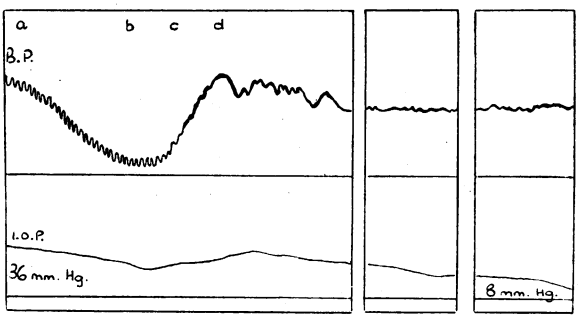

FIG. 8.

Variation of the intra-ocular pressure on increasing the colloid osmotic pressure of the blood.
The upper curve the blood pressure; the lower the intra-ocular pressure. At (*a*) the femoral artery opened; at (*b*) the bleeding stopped; (*c*) injection of 15 per cent. gum arabic; at (*d*) injection stopped. The intra-ocular pressure, after following the blood-pressure initially, tends to fall. (Cat.)

ii. *Intravenous Injection of* 15 *per cent. Gum Acacia.*—Duke-Elder found " the curve of the intra-ocular pressure followed the blood pressure closely at first; but soon after the injection it began to fall steadily independently of the latter." Fig. 8. Duke-Elder's chart of this experiment showed no time marker and the time intervals were not given. It was noted that the intra-ocular tension fell from 36 mm. Hg (the maximum height reached through the blood pressure effect) to 8 mm. Hg (the lowest reading recorded and the finish of the chart). The fall was rapid, and it would appear from the chart that the pressure continued to fall beyond the tenth minute after the injection. When the interchange of fluid (Experiment 3, Table V, p. 414), was studied after a similar experiment it was found that the blood volume reached its maximum ten minutes after the injection, after which fluid began to leave the vascular system for the tissues. If, therefore, the intra-ocular pressure continued to fall for ten minutes, after the termination of the injection, it suggests that the aqueous does not behave like a dialysate, for at the tenth minute fluid had ceased being attracted from the tissues.

There is, moreover, additional proof of the non-dialysate nature of the aqueous humour by comparing the fluid interchange taking place after haemorrhage and the intravenous injection of 6 per cent. or 15 per cent. gum acacia. The tables from experiments No. 2 and No. 3 are reproduced.

TABLE VII

	Blood volume/kg. after haemorrhage and injection of 6 per cent. gum in normal saline (c.cs.)	Blood volume/kg. after haemorrhage after injection of 15 per cent. aqueous gum (c.cs.)
Immediately after -	90	88
5 mins. after -	94	91
10 ,, ,, -	95	98
15 ,, ,, -	95	96
30 ,, ,, -	94	97
45 ,, ,, -	91	92
60 ,, ,, -	88	92
2 hours ,, -	87	89
3 ,, ,, -	87	87

If a comparison is made a marked similarity is noted. After a haemorrhage, the fluid interchange differed very little whether the fluid injected was 15 per cent. gum acacia in water or 6 per cent. gum saline. If, therefore, the eye shared with the other tissues of the body in the loss and gain of fluid, the curves of the intra-ocular pressure in these two experiments should be almost identical. Reference to Duke-Elder's corresponding charts (Figs. 7 and 8) on the curves of the intra-ocular pressure show their complete dissimilarity; after the 6 per cent. gum, the intra-ocular pressure

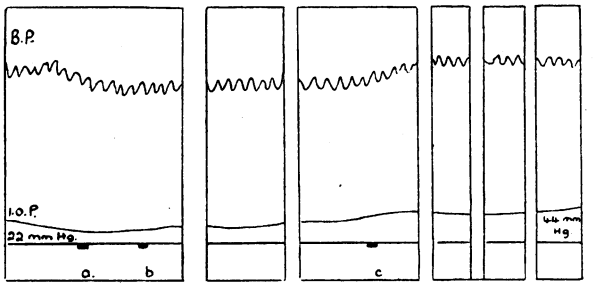

FIG. 9.

Variation of the intra-ocular pressure on decreasing the colloid osmotic pressure of the blood.
The upper curve the blood pressure; the lower the intra-ocular pressure. At (*a*) the femoral artery opened; at (*b*) the artery closed and physiological saline slowly injected; at (*c*) the saline injected rapidly. The intra-ocular pressure, after following the blood pressure initially, tends to rise. (Cat.)

returned to normal, whereas after the 15 per cent. gum, the tension fell steadily to at least only a quarter of the original height.

iii. *Intravenous Injection of Normal Saline.*—Duke-Elder found that the intra-ocular pressure rose steadily from a normal of 22 mm. Hg to 44 mm. Hg (Fig. 9) (*i.e.*, an increase of 100 per cent.). This remarkable rise in the intra-ocular pressure was interpreted by Duke-Elder as being due to the dilution of the blood colloids, fall in osmotic pressure on the blood stream side of the capillary membrane and a forcing out of fluid into the tissue spaces including the eye. When, however, the fluid exchange was studied after haemorrhage and intravenous saline (Experiment 4, Table VI, p. 414), it was found that no water-logging of the tissues took place until two hours after the injection and then the amount of excess fluid in the tissues was negligible. In this experiment, therefore, fluid does not readily leave the vascular system for the tissues, so the enormously increased production of aqueous humour indicated by a 100 per cent. increase in the intra-ocular pressure must be explained by means other than dialysis.

An earlier experiment of Duke-Elder's (1926*a*) has an important bearing on the above experiment (see Fig. 1, p. 31). As a control experiment he injected 30 c.c. of normal saline intravenously into a 3·2 kg. cat and noted that the intra-ocular pressure rose very slightly during the injection, then fell to its normal level. Leathes has studied the fluid interchange in an intact animal after the intravenous injection of normal saline. A summary of his results is :—

TABLE VIII

Time	Volume per kg.
Before - - - - -	70
Normal saline injected at 35 c.c. per kg.	(105—theoretical blood volume)—
Immediately after - - -	80
5 mins. after - - -	79
10 „ „ - - -	78
15 „ „ - - -	77
30 „ „ - - -	75
60 „ „ - - -	71

During the injection 25 c.c. or 5/7 of the volume of fluid injected had left the vascular system for the tissues, 4/5 of the volume had left at the end of ten minutes, and the blood volume was normal at the end of an hour. Thus in an animal not previously bled, intravenous saline rapidly leaves the vascular system for the tissues. If, therefore, the eye shared in the dehydration and water-logging common to all tissues, a gradual and well-marked rise in the intra-ocular pressure would have been expected in such an experiment. Duke-Elder detected none.

Observations have been carried out on the interchange of tissue fluid following the intravenous injections of hypotonic, isotonic and hypertonic solutions of crystalloids and gum. Duke-Elder's records of the variations of the intra-ocular pressure following these injections were in complete conflict with those of the interchange of tissue fluid indicating that the aqueous does not behave like a dialysate.

The Relation Between Depleted Plasma Proteins, the Retention of Fluid in the Tissues, and the Intra-ocular Pressure.—That depletion of the plasma proteins can upset the interchange of fluid has been generally known since Epstein (1914-1926) applied Starling's (1895) views to the oedema of nephrosis. Epstein stated that

consequent upon the massive albuminuria the plasma proteins were reduced to such an extent that their osmotic pressure was no longer able to counterbalance the hydrostatic pressure in the capillaries which forces the fluid from the blood stream to the tissues. Thus more fluid was pushed out into the tissues than could be attracted back again and oedema resulted. Hagedorn, Rasmussen and Rehberg (1925) confirmed this conception of Epstein's by making actual measurements in a case of nephrosis with generalised oedema. They found the capillary blood pressure was somewhat increased to about 150 mm. of water, while the colloid osmotic pressure of the patient's blood was about 100 mm. of water as compared with a normal of 450 mm. There was thus a pressure of 50 mm. of water in favour of filtration from blood to tissues. Further work by Schade and Claussen (1924), Rusznyak (1924), Mayrs (1926), Iversen and Nakazawa (1927-1928), Cope (1928) and Govaerts (1924-1925) amply demonstrated that the osmotic pressure of the serum proteins in normal people lay between 35 and 40 cm. of water and that in nephrotic types with marked oedema the colloid osmotic pressure was uniformly low and under 20 cm. of water. Govaerts (1925) went further and correlated a relationship between the colloid osmotic pressure and the percentage of the individual serum proteins. He found that the smaller serum albumen molecule had a higher osmotic pressure per gram of protein than the larger serum globulin molecule. One gram of albumen per cent. gave an osmotic pressure of 7·50 cm. of water, while one gram of globulin per cent. gave an osmotic pressure of 1·95 cm. of water. Leiter (1928) gave the first direct proof that oedema resulted in intact animals if the plasma proteins were reduced. Oedema was produced in healthy dogs by bleeding them twice daily, 400 to 500 c.c. of blood being withdrawn each time. The blood was centrifuged and the volume of corpuscles suspended in Locke's solution re-injected into the saphenous vein. It was not until the fifth day that oedema appeared at first in the soft tissues of the prepubic region and external genitals, buttocks, thighs, then abdominal and chest walls. At the same time ascites, hydro-thorax, and pulmonary oedema developed. The weight of the dogs increased sharply by 30 per cent. to 40 per cent. in spite of decreased appetite and loss of flesh. The oedema began when the total plasma proteins fell to 3 grams per 100 c.c. or less and disappeared when the proteins rose above that level. Barker and Kirk (1930) confirmed these findings and found that oedema appeared more in relation to the albumen factor. Oedema appeared when the plasma albumen fell to 0·8 gram per cent. and disappeared when it rose to 1 gram per cent., the globulin factor remaining fairly constant about 2 grams per cent. The points of importance in the experimental work of Leiter and Barker and Kirk were :—

1. That oedema could not be produced at the time of the initial experiment.

2. Oedema could appear only if the albumen content of the plasma fell below 0·8 gram per 100 c.c.

The bearing of these conclusions upon Duke-Elder's results is important. A few minutes after his experiment on bleeding and addition of saline in an attempt to reduce the plasma proteins, Duke-Elder obtained a rise of 100 per cent. in the intra-ocular pressure (equivalent to the formation of oedema in the tissue), which demonstrates the eye is not the same as other tissues. But more important in order to produce a rise in the intra-ocular pressure (if the eye was similar to other tissues) the plasma albumen would have to fall to 0·8 gram per cent., and this would require a haemorrhage of at least 4/5 of its blood volume—conditions incompatible with survival from the haemorrhage. In view, however, of the very conclusive rôle the plasma proteins play in the interchange of body fluids, it was decided to investigate the intra-ocular pressure of cases with markedly depleted plasma proteins because—

(1) There is clinical evidence that gross oedema appears when the plasma proteins have become very depleted and their osmotic pressure falls below fifteen mm. mercury through proteinuria, or loss into the alimentary canal.

(2) There is experimental evidence that when the plasma proteins are artificially reduced by bleeding, oedema appears on the fifth day.

In certain types of nephritis such a condition as described above occurs. Consequent upon the massive albuminuria in this disease, the plasma proteins become so reduced that their osmotic pressure cannot counterbalance the hydrostatic pressure of the capillaries and oedema results.

TABLE IX

Name	Intra-ocular tension mm. Hg (Schiötz)	Colloid osmotic pressure	Oedema
S. B - -	20	14	Marked
V. B. - -	23	9	Very marked
E. P. - -	21	8	Very marked
G. L. - -	16	13	Marked
C. C. - -	19	9	Very marked
T. R. - -	22	8	Very marked
E. S. - -	24	10	Marked

Table IX shows the observations made on several cases of nephritis. The intra-ocular pressure was measured in all cases in the morning after the patient had been lying flat for about 20 minutes, with a Schiötz tonometer, and the readings were translated into mm. Hg from a graph provided with the instrument. The colloid osmotic pressure was calculated from percentage of albumen and globulin present in the plasma.

All the cases observed in Table IX had gross oedema, but in no case was the intra-ocular pressure outside the average normal limits. These preliminary observations strongly suggested that the eye could not share in the dehydration and water-logging common to the other tissues of the body, and that the aqueous humour could not be a dialysate. If the aqueous humour were a dialysate, one would have expected a markedly raised intra-ocular pressure in those cases where the plasma proteins were so depleted that gross oedema resulted. It is only possible to explain the above findings on preferential treatment meted out to the eye. It is recognised, however, that the tonometer is an instrument of relative accuracy only, and it was decided to supplement the above observations by a study of one or two cases of nephritis with oedema throughout the course of their illness, and an attempt was made to correlate the variations in the colloid osmotic pressure of the plasma with the degree of oedema and curve of the intra-ocular pressure.

TABLE X

Date	Weight in pounds	Osmotic pressure of plasma proteins mm. Hg	Intra-ocular pressure mm. Hg (Schiötz)	Oedema	Protein in urine (gms per cent.)
28.5.34 -	167	7·9	22	Marked	1·2
7.6.34 -	169	7·5	20	Marked	0·95
15.6.34 -	159	9·6	21	Marked	0·6
9.7.34 -	132	13·8	21		0·6
6.9.34 -	113	15·6	18	Slight oedema	0·5
13.9.34 -	112	16·3	20	Slight oedema	0·55
4.10.34 -	118	19·9	21	No oedema	—
2.11.34 -	126	22·2	21	No oedema	0·40
1.1.35 -	136	25·1	22·5	No oedema	0·10
7.3.35 -	138½	26·4	22	No oedema	—
31.5.35 -	138	25·2	22	No oedema	0·10
15.7.35 -	140	26·7	18	No oedema	0·10
11.10.35 -	138	26·5	21	No oedema	0·06
29.4.36 -	138	24·3	22	No oedema	0·02

The first case studied was Mrs. A. B., aged 31 years. She was admitted to Middlesex Hospital under the care of Dr. Young with gross generalised oedema, ascites and hydro-thorax. Her urine contained over 1 gram per cent. of protein. Under treatment, the oedema gradually subsided.

Weight.—The patient's normal weight was about 136 pounds—and it will be noted that there was a marked gain in weight due to retention of water. As the oedema subsided the weight also fell, until when she was becoming oedema-free the weight had fallen to 112 pounds, indicating quite marked loss of flesh due to illness. The increase in weight from 13/9/34 onwards was unaccompanied by any oedema and indicated marked improvement in health.

The Osmotic Pressure of Plasma Proteins and the Intra-ocular Tension.—The colloid osmotic pressure of the plasma reached as low a figure as 7·5 mm. Hg (as compared with a normal of 33 mm.) and if one assumes the capillary blood pressure to be 11 mm. Hg (as Krogh has recorded in cases of oedema), then in this case there was a pressure head of 3·5 mm. Hg in favour of filtration of fluids from the blood stream to the tissue spaces. On theoretical grounds, therefore, oedema was inevitable and it was present in this case in a very marked degree. If the aqueous humour was a dialysate it should also, like the tissue fluid, give evidence of increased formation, but no increase in intra-ocular pressure was found. This case was followed throughout the period of improvement, and the colloid osmotic pressure of the plasma proteins gradually increased from 7·5 mm. mercury to 26·7 mm. mercury. When the colloid osmotic pressure reached the level of 17 to 19 mm. mercury, the oedema had almost completely disappeared, thus confirming the work of other investigators that there is a critical zone for the osmotic pressure of the serum above which oedema is absent. But no significant change at all could be observed in the intra-ocular pressure throughout the progress of this case, suggesting again that the formation of the aqueous humour could not be explained on physico-chemical lines.

The second case studied was a male aged 27 years. He was admitted to Middlesex Hospital under the care of Dr. Ward with gross oedema and albuminuria.

These results confirm those found in the previous case.

The chemical, osmotic and hydrostatic relationship of the aqueous humour has been carefully reviewed and all the evidence is against the view that the aqueous is formed by dialysis. Several other observations would tend to drive one further and further away from this possibility.

TABLE XI

Date	Weight	Osmotic pressure of plasma proteins mm. Hg	Intra-ocular tension mm. Hg (Schiötz)	Oedema
19.2.35 -	166	7·3	15	Marked
8.3.35 -	156	8·7	17	Marked
14.5.35 -	154	10·6	17	
21.6.35 -	146	10·9	18	Oedema present
12.8.35 -	130½	17·0	15	No oedema
22.10.35 -	128	18·0	17	No oedema
3.6.36 -	126	21·0	15	No oedema
23.11.36 -	128	22·0	17	No oedema

(*a*) Thus Duke-Elder has stated (1926*a*) " If such an eye (an enucleated eye) be placed in isotonic saline or serum it retains its internal pressure for some time and its weight remains constant. If it be placed in hypotonic saline it increases in tension and gains weight initially, while in hypertonic solutions it rapidly becomes of pulpy consistency and loses weight. It is thus clear that the eye is capable of functioning as an osmotic machine."

If the eye functioned like an osmotic machine, a fall in tension would have been expected when it was placed in serum, owing to the osmotic absorption of the aqueous humour by serum.

(*b*) Intravenous hypertonic saline has been given in the preliminary treatment of glaucoma, to bring about a marked fall in the raised intra-ocular pressure. The maximum fall takes place in about six hours and the former tension is usually only regained after seventy-two hours. If the eye behaved like other tissues throughout the body and shared equally in dehydration and water-logging as if the intra-ocular fluids were a dialysate, then the fall in pressure should be greatest at the end of the injection, and at the end of half-an-hour the tension should have returned to its former level.

(*c*) Duke-Elder has stated in his monograph (p. 110), " If for any reason this safety valve action (of the canal of Schlemm) is rendered inefficient or abolished, then disturbances of the equilibrium become cumulative and tend to be permanently effective and the intra-ocular pressure rises." If the aqueous were a dialysate and an interchange of fluid took place all around the surface of the eye, then mere loss of function of the canal of Schlemm, which forms only a small part of the whole surface of the eye, should not cause a rise in the intra-ocular pressure.

(*d*) Priestley Smith's case of intermittent glaucoma (1927)—"A lens imperfectly attached from birth becomes completely detached many years later. One day while the head is bent over the washing basin, the lens passes into the anterior chamber. Pain and high tension quickly follow and advice is sought. The whole pupil is seen through the lens; the peripheral zone of the iris is in contact with the cornea, being driven forward around the lens margin by pressure of fluid imprisoned behind it. Before operation can be undertaken, the lens passes back through the pupil; pain and tension quickly subside. The same displacement recurs several times, each time with a secondary glaucoma and spontaneous recovery. At last, with special precautions against falling backwards of the lens at the moment of operation, the lens is removed and no further glaucomatous attacks occur." . . . And a somewhat similar case by Friedenwald and Pierce (1932*a*), together with the latter's experimental work, demonstrated that re-absorption of aqueous by the tissues behind the iris does not occur.

The Canal of Schlemm.—Very little indeed is known about the nature, structure, function, and content of the canal of Schlemm. Whether it is a capillary, vein or lymphatic channel has never been definitely established.

Structure.—Salzmann (1912) and Maggiore (1917) have described the structures of the scleral furrow of the human eyeball. Schlemm's canal is seldom a single elongated lumen, but is composed of several branches. It has not the structure of a vein, but closely resembles a lymphatic channel. It possesses a very thin endothelial lining with nuclei projecting inwards, and does not lie immediately upon sclera but is embedded in a richly cellular tissue.

Contents.—Direct observation of the irido-corneal angle from inside has assisted a great deal in determining with certainty the contents of the canal of Schlemm. First attempted by Trantas (1907), the chief credit belongs to Salzmann (1914) for giving the examination its present-day value in ophthalmology. Later Koeppe (1919), Uribe-Troncoso (1921-1925), and Barkan (1936) and his colleagues have extended the study of "gonioscopy" as Uribe-Tronsoco named this particular investigation. It is now generally accepted since the introduction of gonioscopy that normally the canal contains aqueous, but that in conditions of stasis it may contain blood due to backflow from the anterior ciliary veins.

Connections.—There is no direct communication between the canal of Schlemm and the anterior chamber, but by means of vessels given off here and there the canal communicates very freely within the sclera with a large closed venous plexus, the "Intrascleral venous plexus," and with the anterior ciliary veins through

their anastomoses with the intrascleral plexus. An actual capillary net for the supply of the canal does not exist. Thus the canal of Schlemm cannot be a capillary, for it possesses no arterial connection, and it cannot be a vein for it has no capillary connections. Further, to be either, its contents must be red blood cells and these have only been observed in the canal of Schlemm during venous stasis.

Pressure Relationships of the Canal and Vessels.—The pressure in the canal of Schlemm has never actually been determined, but many ingenious devices have been employed to assess it.

Duke-Elder has made fresh measurements of the ocular pressures in the dog. Diagramatically he has represented the venous outflow of the dog.

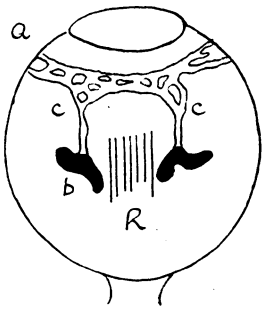

FIG. 10.

The scleral veins of the dog from an injected specimen. (*a*) The circle of Hovius; (*b*) the vortex vein leaving the eye post-equatorially in relation to the rectus muscle R; (*c c*) the anastomosing veins between the two; (*a* and *c*) drawn in open lines—lie within the scleral tissues; (*b*) drawn black come outside the sclera.

The circle of Hovius is a venous plexus which drains blood from part of the choroid, ciliary body and iris. This venous plexus joins the orbital veins and the vortex veins, the latter forming the main venous drain of the eye. The anastomosis of the circle of Hovius with the vortex veins takes place within the sclera. Duke-Elder introduced a canula into one of the scleral veins of the circle of Hovius and noted that the venous pressure was 1 to 2 mm. Hg higher than the intra-ocular pressure in the normal eye. By puncturing one of the retinal veins and noting a haemorrhage into the eye he concluded, since blood flowed out of the vein, the venous pressure must be higher than the intra-ocular pressure. In dealing with conditions of raised intra-ocular pressure where an intra-ocular pressure of 40 mm. Hg was produced, he found the intrascleral venous pressure to be lower by 1 mm. Hg and

the intra-ocular venous pressure to be higher than the chamber pressure. Duke-Elder's findings may be summarised thus:—

VENOUS PRESSURES:

Intra-ocular pressure	Intra-orbital veins	Intrascleral veins
25 mm. Hg	26 mm. Hg	26·5 mm. Hg
40 „ „	41 „ „	39 „ „

It can be seen that the limits within which Duke-Elder worked were very fine and it cannot be wondered at then, that his results are taken with a certain amount of caution. Further, with so important and far-reaching results as he obtained, one would have expected these to be accompanied by an observation on the particular height of the intra-ocular pressure at which the venous pressure reversed its relationship to the chamber pressure. Further, it seems unlikely that two venous systems within the eye should differ by at least 2 mm. Hg. As the intrascleral veins drain the blood from the iris, ciliary body and choroid, it must surely follow that the venous pressure must be higher than the chamber pressure to allow drainage to take place without collapse of these veins. There is, however, a much more serious objection to the pressure relationship as worked out by Duke-Elder. Latarjet (1930) has given an excellent diagrammatic representation of the vascular circulation of the eye. From his diagram it can be seen that the venous return from the ciliary system takes place in two ways—first by the anterior ciliary vein which traverses the sclera, then forms an episcleral plexus, finally to enter the orbital veins and second by branches which join the vortex veins. The canal of Schlemm joins the anterior ciliary veins within the sclera and thus it follows that the pressures in the canal of Schlemm, anterior ciliary vein (intrascleral) and vortex veins (intrascleral) must equal one another and vary the one with the other. Although the blood vessels of the eyeball form two distinct systems—the retinal and choroidal—nevertheless Leber (1876) pointed out that anastomoses take place between the choroidal and retinal systems. More recently Beauvieux and Gouelmino (1924), using very precise methods, have re-studied this problem and they have found that the central vein of the retina has numerous anastomoses with the choroidal and scleral vessels. In view of these anatomical relationships between the vessels of the eye it would follow as a natural conclusion that at all times the pressure in the canal of Schlemm must equal the pressure in the intra-ocular veins and exceed the intra-ocular pressure. In spite of Duke-Elder's very

precise measurements of the pressure within the intrascleral veins, in which he found the intrascleral venous pressure lower than the chamber pressure, the physiological fact remains that blood continued to circulate in the eye, as he himself demonstrated by obtaining a haemorrhage into the eye on puncturing a retinal vein. If the pressure in the retinal veins exceeded the intra-ocular pressure as it must do, then so must also the pressure in the canal of Schlemm.

A further point comparative anatomy also arises regarding these pressure measurements of the canal of Schlemm. By measuring the venous pressures in the circle of Hovius, Duke-Elder states that he has obtained the pressures in the canal of Schlemm under conditions of varying intra-ocular pressures. But is this justified? The circle of Hovius is a venous plexus draining the anterior part of the choroid, the ciliary body, and the iris in direct continuation with the capillaries in these regions. It contains blood and its pressure common to other veins throughout the body is maintained by its capillary connection. The canal of Schlemm on the other hand cannot be a vein, for it has no capillary connections. It normally does not contain blood, and red cells are found only in conditions of venous stasis when back pressure forces blood into the canal from the anterior ciliary veins, its sole anastomosis. The canal of Schelmm must be a specialised structure, but how its pressure is obtained is not clear. It must exceed that in the anterior ciliary veins, else the aqueous could not flow towards the vein, and blood would flow from the vein into the canal.

Functions of the Canal of Schlemm.—The functions of the canal of Schlemm have been variously described and different investigators have held that the aqueous humour can reach the canal by a variety of methods such as pressure filtration, osmotic attraction, by a safety-valve mechanism, and by an active absorption through specialised structures similar to the arachnoid villi.

Pressure Filtration.—This would require the intra-ocular pressure to be greater than the pressure in the canal of Schlemm. Although the pressure in the canal of Schlemm has never been determined, it would seem logical to suppose that it exceeds the chamber pressure. The canal joins the anterior ciliary veins whose pressure it must exceed or blood would enter the canal, and the pressure in the anterior ciliary veins must exceed the chamber pressure, as they anastomose with the choroidal system and this with the retinal system. It would thus seem that a pure pressure filtration of aqueous humour into the canal of Schlemm is impossible even under conditions of raised intra-ocular pressure.

Osmotic Attraction.—The exponents of the dialysation theory must hold that there is a reciprocal ebb and flow in every capillary

throughout the eye as occurs in every capillary throughout the body. If absorption of fluid takes place at the canal of Schlemm, then *ipso facto* it must be a capillary and as such, fluid should pass in the reverse direction and enter the chamber. There is no evidence of the first, and the second is certainly not true. Further, if the canal of Schlemm could attract the aqueous by osmosis it must do so by reason of its protein content, and this in the capillary is provided for by the plasma content of the blood. In no capillary, however, is it possible to have the plasma without the red blood corpuscle. We have seen that normally no red cells have been observed in the canal of Schlemm and this strongly suggests that the canal of Schlemm does not conform to the requirements of a capillary and thus cannot play any part as such in the interchange of fluid by osmosis.

The Safety-valve Mechanism.—Based on pressure relationships in the dog in cases of normal and raised intra-ocular pressure, it has been put forward that when the intra-ocular pressure rises to 40 mm. Hg it exceeds the pressure in the canal of Schlemm and an aqueous humour enters it.

There is, however, a more serious objection to the existence of a safety-valve mechanism. It is known that the enucleated eye has a tension of 8 to 10 mm. Hg which it loses only gradually and a similar state of affairs exists in an animal that has been killed or exsanguinated. In these circumstances when the venous pressure falls to atmospheric pressure, one would surely have expected the aqueous humour to have been filtered off by hydrostatic pressure if a safety-valve mechanism existed. Instead of this, however, the chamber pressure remains at 10 mm. Hg for some time until cellular disintegration takes place. It thus appears that no hydrostatic outflow can take place, and it is a far-reaching hypothesis to say it can take place when the difference of pressure is 1 mm. Hg, when it cannot do so when the difference is 8 to 10 mm. Hg.

Active Absorption of the Aqueous.—As osmosis plays little part in the absorption of the aqueous humour at the angle of the anterior chamber, and as the pressure in the canal of Schlemm makes a hydrostatic outflow impossible, it follows that the intra-ocular fluids can only gain an exit by an active absorption. Two suggestions have been put forward. Thomson (1911) has elaborated the pump-action theory of the scleral spur and has described the establishment at intervals of a negative pressure in the canal of Schlemm following contraction of the ciliary muscle and backward displacement of the scleral process. During this negative pressure phase fluid enters the canal of Schlemm from the angle of the anterior chamber presumably by hydrostatic pressure, and at the same time the change in position of the canal of Schlemm

occludes or kinks its venous connections and back flow of blood from the anterior ciliary veins is prevented. At the end of the negative pressure phase the elastic fibres of the pectinate ligament recoil, the canal of Schlemm returns to its old position, its venous connections become patent and the force of the recoil drives fluid along to the veins.

Wegefarth (1914-1915) considered that the absorption of the aqueous humour and cerebro-spinal fluid were analogous. He has described structures in the dog, bridging gaps in the scleral wall between the ciliary body and Descemet's membrane. These tufts he has maintained resemble and play a part in the passage of fluid similar to the arachnoid villi of the brain and he called them " pectinate villi." He considered that drainage of aqueous did not take place throughout the entire circumference of the angle, but was limited to these irregularly placed " pectinate villi." In the dog there is no canal of Schlemm and the aqueous passes directly from the angle of the anterior chamber into the veins. In the monkey, and man, the canal of Schlemm extends in an unbroken circle and Wegefarth has pointed out that the scleral trabeculae likewise extend completely round the angle and form the inner wall of the canal of Schlemm. He has maintained that the process of elimination of aqueous in man is the same as in the dog, but that the pectinate ligament, instead of appearing irregularly in tufts extends in an unbroken chain around the periphery of the anterior chamber.

The Secretory Theory.—Evidence seems to point to the recently abandoned theory of secretion as being the most likely one in explaining the mechanism of formation of the aqueous humour. It gave way to the theory of dialysis, whose supporters maintained that there was no evidence that work was done or energy was expended in the formation of the aqueous, and that the intra-ocular fluid was in thermo-dynamical equilibrium with the capillary blood. Careful examination of the literature and experimental work forces one to conclude that there must necessarily be an expenditure of energy in aqueous formation. It also shows that there is no chemical equilibrium between the aqueous and the blood plasma and that the aqueous humour does not behave like a dialysate because it fails to respond adequately to variations in the osmotic pressure of the blood.

Conclusions

The views put forward in favour of dialysis for the production of the intra-ocular fluids have been carefully investigated, and it is suggested that the evidence is unconvincing.

There is ample evidence to show that in the production of the aqueous humour there must be an expenditure of energy in the

posterior chamber, for the membrane through which fluid passes into the chamber to become aqueous has an irreversible permeability.

There is evidence to show that there is no chemical equilibrium between the blood and aqueous humour. Easily diffusible constituents such as urea, sugar, uric acid, are not present in equal concentrations in blood compared with the corresponding aqueous.

There is evidence that a physical equilibrium does not exist between blood and aqueous and that the equilibrium level of the intra-ocular pressure is not maintained by the hydrostatic force in the capillaries minus the difference in osmotic pressure between the aqueous and blood.

It is suggested that the evidence chemical, hydrostatic and osmotic points to the formation of the aqueous humour by a process of secretion, and in addition there is evidence of the necessity for the expenditure of energy in its formation.

Summary

The formation of aqueous humour is held to take place by a process of secretion at the ciliary body, and this fluid to move forward to the angle of the anterior chamber and to be actively absorbed into the canal of Schlemm by some process other than osmosis.

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